OECD SEED SCHEMES

GUIDELINES FOR CONTROL PLOT TESTS

AND FIELD INSPECTION OF SEED CROPS

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OECD SCHEMES
FOR THE VARIETAL CERTIFICATION OR THE CONTROL OF SEED MOVING IN INTERNATIONAL TRADE

GUIDELINES

FOR CONTROL PLOT TESTS AND FIELD INSPECTION OF SEED CROPS

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INTRODUCTION

1. The procedures in the following document are guidelines which are advisory to National Designated Authorities (NDA). They are intended to address the methods used to determine varietal purity and identity but not to prescribe the standards and extent of tests or plots which remain the prerogative of the individual NDA.

2. The OECD Schemes for the varietal certification or the control of seed moving in international trade, hereafter called the “OECD Seed Schemes”, are a set of procedures, methods and techniques which verify the quality of seed during the multiplication process and which are designed to ensure that both the varietal identity and the varietal purity of varieties are maintained and safeguarded.

3. Checks are made at different stages of seed production to detect possible mechanical admixtures, mutations, undesired cross-pollination and other unforeseen occurrences that could affect the quality of the seed.

4. In order to do this, the characteristics which distinguish one variety from another have to be established so that it is possible to identify seed crops and seed lots as being consistent with the known characteristics of the variety recognised at the time of Registration. These characteristics are used not only for confirming varietal identity or trueness to type but also varietal purity; they need to be suitable for use in field conditions, although there are also a few characters which in some species relate to the seed itself.

5. The assessment of varietal identity and purity during seed production is crucial for maintaining high standards of seed quality.

6. The seed production process must ensure that nothing happens during the growing of the seed crop, the harvesting operation, the processing, bagging and labelling of the seed lot or the subsequent distribution which could adversely affect seed quality.

7. The OECD Seed Schemes provide procedures which are designed to check the progress of a variety at different stages in the seed production process. These are as follows:

- Examination of control plots using samples of seed drawn from lots;
- Laboratory tests on seeds and seedlings, using samples of seed drawn from lots;
- Field inspection of growing seed crops, on one or more occasions.

8. In these tests and inspections it is essential to adopt technical methods which achieve sufficiently accurate and reliable results, yet can be operated within the limits of available resources. The approval of seed multiplication is based on control plot, field inspection or laboratory tests, or a combination of two or three of these.

9. The methods described in these Guidelines have been found, over a period of many years, to give satisfactory results and provide the principles upon which such methods should be based. They have been adopted by most OECD Member and Non member countries participating in the OECD Seed Schemes and exchanging certified seed in international trade.

PART I. CONTROL PLOT TESTS

Purpose

10. Control plot tests are used to monitor the identity and purity of a variety (hybrid or non-hybrid) at various stages in the seed multiplication programme, thereby assuring the National Designated Authority that the quality of seed produced in the OECD Schemes is of a satisfactory level.

11. The control plot tests are designed to answer two questions:

- Does the sample generally conform to the description of the variety, thereby confirming its identity?
- Does the sample conform to the published standards for varietal purity?

12. The first question can be answered by making a visual comparison between the control plot, sown with a sample of seed representative of the seed lot, which should be drawn by an official or an authorised sampler, and a plot grown from a reference sample, hereafter referred to as the "Standard Sample".

13. The second question requires the identification of off-type plants within the control plot so that their numbers can be related to the standards published in the OECD Seed Schemes. This test measures the uniformity of the seed lot and determines whether the characteristics of the variety have remained unchanged during seed multiplication. It will also indicate the effectiveness of the Scheme and of limiting the number of crop generations.

Pre-control

14. Pre-control is the term applied to variety verification of early generation seed, i.e. Pre-basic and Basic seed. When an early generation seed lot is being multiplied to produce a further generation of seed, the information provided by a control plot is invaluable in that it gives the National Designated Authority data on identity and quality that are available before – or about the same time – as the next seed crop is ready for field inspection. In this instance the test, which is referred to as a pre-control test, is grown simultaneously with the seed crop of the following generation. Pre-control is a very important component of a seed multiplication and certification programme because of its ability to identify varietal identity and varietal purity insufficiencies at an early stage, before they become a major widespread problem. The pre-control is very reliable and for many species the only tool for the assessment of varietal identity. In addition, the pre-control generates useful information concerning varietal purity, seed-borne diseases etc. that can support the field inspection of the corresponding multiplications.

15. Although field inspections are an essential requirement of OECD Seed Schemes, there are many advantages available to National Designated Authorities in conducting Pre-control plots. These are as follows:

- Plants representing the seed lot of the variety can be observed as frequently as necessary.
- The observation period can be extended from seedling emergence to full maturity.
- All plants in the control plot population can be examined in detail if necessary.
- A comparison can be made with the Standard Sample.

- Comparisons can also be made with seed lots of the same variety in the same and previous generations.
- One expert can make judgements on all control plots for all varieties and categories thus ensuring the standardisation of recording.
- Where the land is free from volunteers and clean machines have been used for sowing, the National Designated Authority can be certain that all off-type plants observed in the control plot have arisen from the seed sample.
- National Designated Authorities may use an adverse pre-control plot test result to reject seed crops sown with the same seed lot.

Post-control

16. Post-control is a term normally applied to variety verification of Certified Seed which is not further multiplied. In the year that the plots are being grown, the Certified Seed has been sold to farmers and planted for production, and test results will come too late for remedial action unless the seed lot – or parts of it – was not marketed. It is called post-control, because the result is not available until after the seed has been certified. Post-control tests are nevertheless valuable, because they monitor how efficient the seed production process has been in maintaining varietal purity and identify ways in which the system might be improved. By allowing comparisons between plants grown from the seed lot produced and those grown from the Standard Sample, the National Designated Authority can monitor quality and give assurance that the minimum standards are being upheld.

17. For Certified Seed that is to be further multiplied, e.g. C1 seed being multiplied to produce C2 seed, one control plot can serve two functions: that of post-controlling the C1 seed lot from the last harvest and pre-controlling a C2 seed crop for the next harvest.

18. In the case of hybrid varieties, because the varietal identity and purity of the hybrid cannot be verified in the seed production field, it is necessary to assure production quality in the post-control plots.

19. The hybrid variety observed in post-control plots must be true to its varietal identity, and the plants must conform to the characteristics of the variety listed by the National Designated Authority at the time of its registration.

Off-season control

20. In order to obtain results from observations in control plots without waiting for the end of the following growing season, it is possible to conduct these tests (pre-control and post-control) in a region of another hemisphere. In this way the quality of the basic seed, of hybrid parental components and of non-commercialised seed in stock can be determined before the following sowing period.

Standard Sample

21. Varietal identity and purity checks in pre- and post-control can be done best by comparing plants grown from a sample of the seed lot with plants grown from seed of the “Standard Sample”.

22. The purpose of the Standard Sample is to provide a living description of the variety; its supply, maintenance and authentication are critical.

23. It is important to take into account that there are often two official reference samples held by the authorities responsible for registration and certification.

24. The first is the sample which is used by the authority in charge of the national list of varieties. When a new variety is submitted for registration, the Listing Authority uses a sample as the official standard in tests for assessing distinctness, uniformity and stability (hereafter called "Definitive Sample"). It is the Listing Authority which retains and uses the Definitive Sample primarily for registration purposes. The sample should be sufficiently large to satisfy requests for small quantities of seed both from within the participant country and also from other Listing Authorities. In some cases it may be difficult to satisfy requests for seed from National Designated Authorities for certification purposes, since the quantities required may be large and would therefore use up the Definitive Sample too quickly.

25. The second reference sample is the Standard Sample. It is used by the National Designated Authority as the official standard in pre- and post-control plots against which all other samples of seed of the variety in seed certification are judged for trueness to variety. It is the National Designated Authority which retains and uses the Standard Sample of listed varieties specifically for use in seed certification. Before use, the Standard Sample should be checked and verified by both the Listing Authority and the Designated Authority to ensure that it is authentic and identical to the Definitive Sample.

26. The Standard Sample should be obtained by the National Designated Authority directly from the breeder or the maintainer. Alternatively, where relatively large amounts of seed of the Standard Sample are required – not only for the annual establishment of control plot tests of certified seed but also to meet requests for seed from other National Designated Authorities – it is permitted to use a sample from a Pre- basic seed lot which has been checked for uniformity and trueness to variety against the Definitive Sample.

27. In the case of synthetic varieties of allogamous species and all hybrid varieties, it is the final generation of certified seed which will constitute the Standard Sample. For certain species and for hybrid varieties it may be necessary to have separate Standard Samples that represent the inbred lines and parental components which are used at the Basic and Pre-basic seed level to produce the hybrid variety.

28. Some countries may use the Definitive Sample in place of the Standard Sample for seed control plot tests, and in these cases the Definitive Sample has a dual function. However, this will only be practicable where the demand for certification control purposes is small.

29. In the case of Standard Samples of varieties originating from the country of another National Designated Authority, it is essential for the Standard Sample to be obtained from that Authority and not directly from the breeder.

30. The Standard Sample, which is recognised as truly providing a living description of the variety during the test period, is the most reliable standard by which seed certification samples can be judged. It should be used in conjunction with the official description, bearing in mind that the description of varieties may have some limitations since it is not always sufficiently precise for the purpose of classifying and identifying varieties.

31. When the germination of the Standard Sample begins to fall or the stock of seed needs replenishing, a new sample should be requested. There must, however, be sufficient

time for the comparison of the new sample with the old sample in a field test for at least one cropping season in order to check its authenticity and before the original Standard Sample is discarded.

Previous cropping

32. In siting control plots, the National Designated Authority, or its agent, must ensure that the field is suitable. There must be no risk of contamination from volunteer plants of the same or closely related species or similar crop groups. This is done by checking the previous cropping of the field to be used and ensuring that a carefully planned rotation has allowed the field to be cleaned of seeds – shed by both crop plants and weed species – after the harvest.

33. Attention should be paid to seeds of species that are known to remain dormant in the soil for a number of years or which are prevented from germinating before being cultivated into the soil. The seed of a number of species of crop plants can survive in the soil for several years, if the conditions are suitable. Seeds with high oil content such as those of Swede rape (*Brassica napus*) and Turnip rape (*Brassica rapa*) are known to remain viable for many years. There is also evidence that seeds of small grained cereals can survive for several years when conditions are favourable. Once control plots of these species have been recorded for varietal identity and varietal purity, it is recommended that they are destroyed before the seed that has been set is viable. This reduces the likelihood of volunteers being present in the field the next time the field is used to grow control plots of these species.

Husbandry

34. A good uniform seed bed is desirable to promote the rapid and uniform establishment of control plots.

35. Husbandry requirements for control plots are usually similar to those for commercial crops with the exceptions that variety differences and characteristics should be maintained whenever possible, and the condition of the plots should permit examination throughout all the relevant growth stages. It may be necessary to keep fertiliser levels at a minimum in order to avoid lodging, especially in cereal crops.

36. Care should also be taken in the use of herbicides and plant growth regulators which could affect the morphology of the plant.

Control plot layout

37. The control plot tests should be designed in such a way that observations can be easily made.

38. A simple layout with all samples of the same variety grouped together will provide the best basis for comparison with the Standard Sample (this is also true for the hybrid variety components). It is also an advantage to put similar varieties in close proximity to highlight differences that exist between them.

39. Within a particular variety, recording is facilitated if related seed lots, which share the same antecedent, are sown in neighbouring plots. In this way contaminants seen in one plot can be readily examined for their presence in adjoining plots.

40. The plots should be duplicated in another part of the field whenever possible and if resources permit, so that additional data can be obtained. For some categories of seed replication may be essential in order to achieve the minimum number of plants for recording. For some species of grasses and fodder legumes it may be necessary to use a spaced plant design for control plots to allow measurements of morphological characters such as leaf length, leaf width, plant height etc. to be made on single plants.

41. The design of the test should enable appropriate statistical analysis of the results and decision-making on the basis of conventional confidence limits.

Recording

42. Recording of the control plots should start when plants reach growth stages at which varietal characteristics can be observed. Depending on the species this can be during vegetative growth stages, at flowering or at full maturity. Control plots can also be recorded for species purity and for the presence of seed-borne diseases.

43. The latter part of this document includes the main characteristics that may be used in control plot tests. For many species they are based on the characters included in the UPOV Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability, and they are divided into "primary" and "secondary" characteristics for OECD Seed Schemes' purposes.

44. In the determination of varietal purity, the standards are expressed either as a percentage of the population or where plant populations are not easily determined, e.g. grass species, as a number per unit area. The off-type plant count in the plot can be used to give a probability of the seed lot meeting published standards, provided the plot size is sufficiently large. Reject numbers should be used which relate the number of off-type plants observed in a sample to a published standard in such a way that reasonable account is taken of the risks of incorrect acceptance or rejection of the seed lot. The degree of risk is related to the sample size.

45. Recording varietal or species purity – or levels of seed-borne disease – involves the identification of plants which are different in appearance. Plants which are atypical for major plant characters should be examined in more detail. A method of recording the individuals and identifying them is required so that they are not counted twice on future visits. Labels, markers or coloured wool have been used successfully for this purpose.

46. The average plant population of each control plot, for those species where the varietal purity standards are expressed as a percentage, should be estimated in order to facilitate the calculation of the level of varietal impurities. Where the number of off-types in a plot is close to or in excess of the likely reject number, the plot population should be more accurately assessed to ensure accuracy in determining the number of plants examined.

47. It is usually possible to see when a plot has the wrong identity or is badly contaminated with an off-type plant. It may, however, be difficult to decide whether an individual plant should be classed as an off-type. Such decisions require the experience of a plot recorder, who is an expert in the examination of the morphological characteristics of the species under study and also has detailed knowledge of the characteristics of the variety.

48. "Subjective" judgements must be made with the help of the variety description as to whether the off-type is a genetic variant or whether a normal variation between plants has been exaggerated by environmental factors. In general terms, the recorder should ignore

small variations and only include clearly distinct off-types in the final count which may determine acceptance or rejection of the sample.

49. In the case of male sterile hybrid component, in addition to the varietal purity assessment, all the plants of the plot should be carefully checked to determine if any are producing viable pollen.

50. Particular case of hybrid rye *(Secale cereale)*. Hybrid rye seed production involves the physical mixing of a restoring pollinator with a single-cross hybrid. The sample drawn from the resulting three-way cross hybrid production is consequently a mixture of the hybrid seeds and of self-pollinated pollinator seeds. This must be considered when counting impurities in the post-control plots.

Reject numbers

51. Reject numbers relate the number of off-type plants observed in a sample to a published standard. In order to decide whether the varietal purity of a control plot is within a given standard, the number of off-types in the plots is counted. The plants in the control plots (of limited size) may, due to sampling errors, not have the same frequency of off-types as the corresponding seed lot.

52. The sample sizes or plot areas for examination need to be sufficient to make reject numbers valid. For pre-control of certain species this may not always be feasible, because it would require considerable labour and land resources and would be very expensive.

53. Reject numbers can be used for measuring the quality of the certified seed that has been marketed. The reject numbers in tables 1, 2 and 3 in the following articles apply to control plots. Tables 1 and 3 are applicable only when plants are counted. If ears are counted then larger reject values are required and table 2 should be used.

54. In order to lower the risk of classifying a control plot as not satisfying the given varietal purity standards, it is necessary to accept more off-types than specified by the standards. However, increasing the number of off-types allowed will also increase the risk of erroneously classifying a post control plot as meeting the varietal purity standards. There are therefore two different types of risks in the assessment:

- The risk of classifying as unsatisfactory a seed lot that satisfies the varietal purity standard (the α or producer risk).
- The risk of classifying as satisfactory a seed lot that does not satisfy the varietal purity standard (the β or consumer risk).

55. It is necessary to decide to what degree to limit the risk of incorrectly classifying a post control plot.

56. A set of “reject tables” is used rather than a straightforward application of the standard. The standards are converted into reject values at α=0.05 using the binomial probability distribution. A sample is considered to be non-conforming to the standard – and rejected – if the number of off-type plants is equal to or greater than the reject number for a given population.

57. In choosing a sample size or plot area for examination, the costs and time involved in observing large samples have to be balanced against the risks of making a wrong decision. As a general rule, a sample size of 4 x n can be used when the standard level to be applied is 1 for n.

58. Table 1 below provides the plant reject numbers for various sample sizes and standards.

- Example: For a varietal purity standard of 99.9%, i.e. an impurities threshold of 1 per 1 000, the rejection rule (i.e. 9 or more off-type plants out of a sample of 4 000 plants observed) limits the risk of incorrectly rejecting a seed lot to 5% ($\alpha < 0.05$).
- Note: It should be noted that at this probability level (95%) the system is biased in favour of the seed producer, since the risk of an incorrect acceptance of a seed lot is higher than the risk of an incorrect rejection.

59. In Table 1, reject numbers with a white background are not as reliable as those with a grey background, because the sample size is not large enough, and there is a greater risk of an incorrect acceptance of unsatisfactory seed lots.

Table 1. Reject numbers for various sample sizes and varietal purity standards ($\alpha < 0.05$)

Sample size *(plants)*	Varietal purity standard				
	99.9%	99.7%	99.5%	99.0%	98.0%
	Reject number *(plants)*				
200	--	--	4	6	9
300	--	--	5	7	11
400	--	4	6	9	14
1 000	4	7	10	16	29
1 400	5	9	13	21	38
2 000	6	11	16	29	52
4 000	9	19	28	52	96

Note: The symbol (--) indicates that the sample size is too small for a valid test of the sample.

60. Table 2 below provides the ear reject numbers for various sample sizes and standards

Table 2. Reject numbers for various sample sizes and varietal purity standards ($\alpha < 0.05$)

Sample size *(ears)*	Varietal purity standard				
	99.9%	99.7%	99.5%	99.0%	98.0%
	Reject number *(ears)*				
200	--	--	5	7	11
300	--	--	6	9	14
400	--	5	7	11	17
1 000	5	9	12	20	34
1 400	6	11	16	26	44
2 000	7	14	20	34	59
4 000	11	23	34	59	106
8 000	17	39	59	106	197

Note: The symbol (--) indicates that the sample size is too small for a valid test of the sample.

Control plot tests for species where the varietal purity standard is expressed as a number per unit area

61. For a number of species, the varietal purity standard is expressed as a number per unit area, because determining the number of plants per unit area is very difficult, if not impossible, for these species.

- For example, the varietal purity standards applied in *Lolium perenne* seed crops are as follows:
- The number of plants of *Lolium perenne* which are recognisable as being not true to the variety concerned shall not exceed one plant in 50m^2 in crops to produce Basic Seed and one plant in 10m^2 in crops to produce Certified Seed. (OECD Grass and Legume Seed Scheme, 2012, Appendix 1, par. 7.2)

62. In the case of a post control test of *Lolium perenne*, it would – in theory – be necessary to examine an area of 120m^2 to verify the varietal purity of Basic Seed and 40 m^2 when verifying Certified Seed. As the plant density in the control plot is often quite different from that in the multiplication field, it is very difficult to reliably assess the varietal purity of a control plot to the field standard.

63. In order to obtain some information on the varietal purity level of a given post control plot, it is recommended that an area of at least 5m^2 is examined. For that the following reject numbers should be used in order to determine whether the varietal purity of a post control plot gives cause for concern.

Table 3. Reject numbers for various areas examined and varietal purity standards (α <0.05)

Area of plants examined (m^2)	Varietal purity standard					
	1 /50m^2	1 /30m^2	1 /20m^2	1 /10m^2	4 /10m^2	6 /10m^2
5	2	2	2	3	6	7
10	2	2	3	4	9	11
15	2	3	3	5	11	15
20	3	3	4	6	14	19
25	3	4	4	6	16	23
30	3	4	5	7	19	26
35	3	4	5	8	21	30
40	3	4	6	9	24	33
45	4	5	6	9	26	37
50	4	5	6	10	29	40

64. The Designated Authority can decide on the plot size/area to be examined.

Post-control plot tests for Maize

65. In the case of open-pollinated varieties of maize, the varietal purity standards applied in the field crop are as follows (OECD Maize and Sorghum Seed Scheme, 2012, Appendix 1, par. 5.1 and par. 9.1):

1. In crops to produce Basic Seed, the minimum varietal purity is 99.5% (=maximum of 1 off-type plant in 200 plants);

2. In crops to produce Certified Seed, the minimum varietal purity is 99.0% (= maximum of 1 off-type plant in 100 plants).
3. In crops to produce Basic Seed of parental lines of hybrid varieties of maize, the minimum varietal purity is 99.9% (= maximum of 1 off-type plant in 1 000 plants).

66. In the case of the post-control of Certified Seed lots of hybrid varieties of maize, the varietal purity standard for single cross hybrids is 97.0% (= maximum of 1 off-type plant in 33 plants). The varietal purity standard for other types of hybrid is 95.0% (=maximum of 1 off-type plant in 20 plants). These standards are included in the 2012 Rules of the OECD Maize and Sorghum Seed Scheme.

Applying the actual varietal purity standard to plots

67. In view of the fact that the varietal purity standards prescribed for Certified seed lots of hybrid varieties in post control are relatively low at 97.0% for single cross hybrids and 95.0% for other types of hybrid, the National Designated Authority may wish to apply the actual varietal purity standard to determine whether or not the seed lot is satisfactory in post-control plots (Tables 4, 5 and 6).

Table 4. Applying actual standard for varietal purity standard of 97.0%.

Single cross hybrids

Sample Size - Number of Plants	Pass	Fail
100	3	4
67-99	2	3
33-66	1	2
<33	0	1

Table 5. Applying actual standard for varietal purity standard of 95.0%.

Three-way cross, top-cross and other hybrids

Sample Size - Number of Plants	Pass	Fail
100	5	6
80-99	4	5
60-79	3	4
40-59	2	3
20-39	1	2
<20	0	1

Table 6. Applying actual standard for purity standards of 99%, 99.5% and 99.9%

Sample Size - Number of Plants	Varietal Purity –Applying the actual standard to plots					
	Basic Seed Hybrid		Basic Seed Open-pollinated		Certified Seed Open-pollinated	
	99.9%		99.5%		99.0%	
	Pass	Fail	Pass	Fail	Pass	Fail
<1000	0	1	-		-	

200	0	1	1	2	2	3
100	0	1	0	1	1	2
75	0	1	0	1	0	1
50	0	1	0	1	0	1

Applying reject numbers to maize plots

68. Reject numbers based on the numbers given in Tables 7, 8 and 9 can be used in addition to the actual standard for determining whether seed lots of both open-pollinated and hybrid varieties meet the required varietal purity standards in post-control for various sample sizes. The application of reject numbers introduces a calculated allowance for sampling error. One effect of sampling error is for a limited sample to contain proportionately more contaminants than the seed from which it is taken. In a system where the number of plants that can be reasonably included in a post control plot is limited by resources, an indication of the likely error is valuable for deciding on the action to be taken. It may, however, be pragmatically accepted that the application of the standard itself achieves the aim of ensuring seed quality, albeit with a higher number of lots being rejected. As a general rule, and within reason, the greater the number of plants that can be grown in post control plots, the more accurate is the indication of off-type levels in the original seed lot.

Table 7. Reject numbers for various samples sizes for varietal purity standards of 99.9%, 99.5% and 99.0%. (α < 0.05)

Sample Size - Number of Plants	Varietal Purity		
	Basic Seed Hybrid	Basic Seed Open-pollinated	Certified Seed Open-pollinated
	99.9%	99.5%	99.0%
100	--	3	4
200	--	4	6
300	--	5	7
400	--	6	8
500	3	7	10
600	3	7	11
700	3	8	13
800	3	9	14
900	4	9	15
1 000	4	10	16
1 100	4	11	18
1 200	4	11	19
1 300	4	12	20
1 400	5	13	21
1 500	5	13	23
1 600	5	14	24
1 700	5	15	25
1 800	5	15	26
1 900	5	16	27
2 000	6	16	29

Table 8. Reject numbers for various sample sizes for a varietal purity standard of 97.0%,

Single cross hybrids (α < 0.05)

Sample Size - Number of Plants	Varietal Purity 97.0%
47-66	5
67-88	6
89-110	7
111-134	8
135-158	9
159-182	10
183-207	11
208-232	12
233-258	13

Table 9. Reject numbers for various sample sizes for a varietal purity standard of 95.0%,

Three-way cross, Top cross and other hybrids (α < 0.05)

Sample Size - Number of Plants	Varietal Purity 95.0%
41 – 53	6
54 – 67	7
68 – 81	8
82 – 95	9
96 – 110	10
111 – 125	11

PART II. FIELD INSPECTION OF SEED CROPS

Purpose

69. Field inspection of growing seed crops is the second procedure required by the OECD Seed Schemes. The most important functions are to check that the seed crop shows the characteristics of the variety which it claims to be (varietal identity) and to ensure there are no circumstances which might be prejudicial to the quality of the seed to be harvested (varietal purity).

70. Seed crops may be inspected frequently during the growing season. There must be at least one inspection which is timed to allow the best opportunity to assess varietal identity and purity, but there may be more.

71. With many crops the ideal time for field inspections is during the flowering period or immediately before dehiscence of the anthers. With some crops a vegetative inspection is also required and with others observations at full maturity are essential.

72. Although the technique of field inspection differs in detail, depending on the particular features of each species, the main principles for field inspection checks are as follows:

- The previous cropping history of the field should be such that the risk of undesirable volunteer plants of the same or related species contaminating the seed crop is reduced to a minimum.
- The seed crop should be sufficiently isolated from other crops to reduce the risk of contamination with undesirable pollen.
- The crop should be physically isolated to prevent mechanical admixture at harvest.
- The seed crop should be isolated from sources of seed-borne disease.
- The seed crop should be reasonably free from weeds and other crop species, especially those whose seeds may be difficult to separate from the seed crop during seed processing.
- The seed crop should have the correct varietal identity.
- There should not be more off-type plants present than the varietal purity standards allow.
- There should not be more plants of other species present than the standards allow.
- For hybrid varieties the proportion of male to female plants should be satisfactory and as defined by the maintainer. The physical or genetic emasculation of female seed-bearing plants should be effective.

Principles

73. The person conducting the field inspection should be provided with all information about the seed crop. The inspector should be an expert in recognising the characteristics of the species, which are used for distinguishing varieties, and have a sound knowledge of the varieties to be inspected. The information provided should include a description of the variety, or of the parental lines/components in the case of hybrid production. The inspector should also be informed of the history of the seed used to sow the seed crop, together with

results from the pre-control plot grown concurrently by the National Designated Authority. The cropping history of the field for the past five years should also be available to the inspector.

74. The inspector is required to give an independent opinion of the seed crop and is responsible to the National Designated Authority. The function of the inspector is to report the state of the crop at the time of inspection. The timing of the inspection may be such that some off-types may be hidden or difficult to identify. In this case a second or subsequent inspection might be required before a decision can be made.

75. The inspection of the seed crop should be supplemented by results from the pre-control plot, which the National Designated Authority has under continuous observation and which will provide the inspector with reliable data on all varietal identity and purity aspects relevant to the seed lot(s) used.

Previous cropping

76. The crop inspector should interview the grower of the seed crop concerning details of the previous cropping of the field. The grower should provide details relating to the crops grown on the field for the previous five years. Information about possible a sub-division of a field in previous years, or any previous cropping with the same variety, can also be established at this time.

77. In the case of hybrid production, the same field cannot be used consecutively for the same species, to avoid the growth of fertile volunteers from hybrid seed production of previous years.

Authentication

78. In order to authenticate the identity of the seed sown, growers should retain at least one label from each seed lot used to sow the crop. A second label of each seed lot used should be displayed in the field by the grower where it can be clearly seen by the inspector.

79. For hybrids, labels of the seed lots used for male parent and female parents must be kept and verified.

80. The purpose of this procedure is to check the details provided on the label against those on the crop inspection form, and to confirm the identity of the variety.

Varietal identity

81. The first function of the field inspection is to establish that the seed crop as a whole is consistent with the characteristics of the variety given in the official description. This is usually done by walking into the seed crop and examining a reasonable number of plants. The actual number to be examined in each case will depend on the complexity of the distinguishing characters and the uniformity of the variety. Thus it would be necessary to examine a larger number of plants for allogamous species than for autogamous species.

82. For some species, positive confirmation of identity of individual varieties may not always be possible in the seed crop, but it should always be possible to ensure that the crop is in the correct variety group. Access to the control plots will familiarize the inspector with the characteristics of the variety and be aware of the differences with other varieties in the same variety group.

83. In the case of hybrid varieties, the inspector must be able to identify without difficulty the male parental line and the female parental line. The inspector must check the varietal identity of each parental component using the corresponding official descriptions.

Condition of the seed crop

84. After having examined the field as a whole, the inspector should examine the field in more detail, especially around the perimeter.

85. Observations of signs that indicate that part of the field might have been sown with different seed or might have become contaminated should be made; for instance, in field gateways or on headlands. Places in the field where sowing started should be located to check that the drilling equipment had been properly cleaned before sowing. Particular attention should also be paid to the presence of other crop species, weeds, seed-borne diseases, and verification of isolation from sources of contaminating pollen.

86. The general appraisal of the seed crop should determine whether it is in satisfactory condition to permit the detailed examination of plants for varietal purity.

87. Crops which are severely lodged, badly infested with weeds, stunted or poorly grown because of disease, pests or other causes and which cannot be assessed for varietal purity should be rejected. The inspector, however, could use the evidence of the pre-control plot to supplement the information in the field in intermediate cases.

Isolation

88. Isolation of the seed crop should be checked whilst walking around its perimeter. For crop species which are cross-pollinated by insects or wind, this will involve checking all surrounding fields for any crops lying within the minimum prescribed isolation distances that might cross-pollinate with the seed crop.

89. Where the isolation distance between the hybrid seed crop and a source of contaminating pollen is insufficient to satisfy the minimum requirements, the inspector must request partial or total destruction of the contaminating source so that the desired isolation distance is met.

90. When isolation is satisfied by the existence of a pollen barrier of the male parent of the hybrid around the crop to produce the hybrid seed, the inspector must be assured of the coincidence of flowering between the male and female parents.

91. The minimum isolation distances are given in the OECD Seed Schemes.

92. A map of the seed crop and the surrounding crops, provided by the grower, should alert the inspector to potential sources of foreign pollen.

93. The inspector should also look for volunteer plants or weeds, both in the seed crop and neighbouring crops, which could also be a source of contaminating pollen. In the case of seed production of hybrid Sorghum spp, the inspector should look for any potential plants of other sorghum species [in particular Aleppo grass (*Sorghum halepense*)].

94. Additionally, seed crops of autogamous species and apomictic varieties of Smooth-Stalked Meadowgrass (*Poa pratensis*) should be isolated from other crops by a definite barrier or a space sufficient to prevent physical admixture during harvesting.

95. Checks should also be made to ensure that seed crops are isolated from other crops which may be infected with seed-borne diseases.

Species purity

96. For many crop species, the rules of the Schemes do not include species purity standards to be applied in seed crops.

97. Nevertheless, for some crop species, there are minimum species purity standards in addition to those for varietal purity, and these must be assessed at the time of crop inspection.

- Grass and Legume Seed Scheme: See Annex VI, Appendix 1, par. 7 for *Lolium* and for other species;
- Seed Scheme for Crucifer and other Oil or Fibre Species: See Annex VII, Appendix 1, par. 7;
- Maize and Sorghum Seed Scheme: For non-hybrid varieties of *Sorghum bicolor* and *Sorghum sudanese* see Annex XI, Appendix 1, par. 6. For Sorghum spp. varieties, see Annex XI, Appendix 1, par. 10.

98. In the case of seed crops that produce hybrid varieties of Sorghum spp., they shall not contain more than 1 plant in 10 m^2 of another species of sorghum, the seeds of which are difficult to distinguish in a laboratory test from the crop seeds or which will readily cross-pollinate with the crop being grown for seed.

99. If there are varietal impurities in addition to plants of other species, the inspector should apply the appropriate varietal purity standard separately, the procedures for which are given below.

100. Nevertheless, the presence of a number of crop species and weed species in a seed field can create problems not only in the seed crop but also in the processing of the seed.

101. In particular, the following are recognised as causing difficulties in some countries:

- Ryegrass (Lolium spp) and cereals in seed crops of other cereals species;
- Field pea (*Pisum sativum*), field bean (*Vicia faba*) and french bean (*Phaseolus vulgaris*) in seed crops of lentil (*Lens culinaris*) and chickpea (*Cicer arietinum*);
- Cow pea (Vigna spp) in field pea (*Pisum sativum*).

102. There are also a number of weed species which can prove difficult to clean from seed during seed processing. The following associations have been identified in various countries:

- Ryegrass (Lolium remotum) in linseed (Linum usitatissimum);
- Wild mustard (charlock) (Sinapis arvenis) in white mustard (Sinapis alba), Swede rape (Brassica napus), Turnip rape (Brassica rapa) and brown mustard (Brassica juncea);
- Aleppo sorghum (Sorghum halepensis) in hybrid sorghum;
- Wild oats (Avena fatua) in cereals.

Varietal purity

Requirements for all crops

103. Assuming that crop location, authenticity, varietal identity, isolation and crop condition are all satisfactory; the final stage in the inspection is the assessment of varietal purity.

104. To do this, it is necessary to follow a sampling procedure which focuses attention on small areas of the seed crop for detailed examination.

105. The number and size of these areas have to be related to the specific minimum varietal purity standards relevant to the crop species and the category of seed being produced.

106. When deciding how many sample areas should be examined, it is necessary to balance the requirements for statistical accuracy and the need for reasonable confidence in the result with the time available for making inspections. This may involve a compromise in favour of a reduced workload for practical reasons and, consequently, to an increased risk of making the wrong decision. Generally, there is a bias in favour of accepting a crop which may have an impurity level greater than the desired standard. However, this can be justified since the standards for varietal purity are normally higher than is strictly necessary for commercial crop production.

107. The location of the sampling areas should be such that the whole field is effectively covered, and the inspector should follow a pre-defined procedure. This, however, may have to be adapted to the shape and size of each field, to the particular features of each species, but in particular whether the standard for varietal purity is expressed as a percentage or as a maximum number of off-types per unit area.

108. The distribution of the sampling areas should be random and widespread so as to represent the whole crop. There should be no conscious selection of areas which appear to be any better or worse than the average of the crop. This can be achieved in practice by deciding on a pre-determined distance between each sample area. The direction of drilling should also be taken into account so that each sampling area includes a different pass of the seed drill.

109. The fourth part of the present document "*Characteristics for assessing varietal purity*" provides for each species those morphological and physiological characters which have been found to be of greatest use in distinguishing between varieties, thus finding the off-type plants (varietal impurities). The varietal impurities may include other identifiable varieties, deviant plants or various varietal types.

110. The ease of identifying impurities will differ considerably by seed crop. Differences such as height, colour, shape, maturity are clearly identified. Less obvious impurities, for example leaf shape, leaf hairiness, flower and seed characters, may only be detected by checking a particular part of the plant. Larger samples can be examined for obvious impurities than for those which are less obvious, and these should be taken at random and from as wide an area of the field as possible.

111. The results of the relevant control plot corresponding to the basic seed lots used should be made available to the inspector. This will enable off-types, found in the pre-control plot by the National Designated Authority, to be positively confirmed in the seed crop. There may also be off-types that are present in the seed crop but which were not

observed as occurring in the control plot; these too need to be recorded and taken into account when determining whether the crop is acceptable.

Additional requirements for hybrid crops

112. When inspecting crops to produce hybrid varieties, the inspector must be assured, before verifying the varietal purity of the male and female components, that there has been no accidental mixing of the two component rows.

113. In the case of maize, sorghum and sunflower hybrid seed production, purification by roguing is an acceptable method for obtaining varietal purity for one or other of the two parents. In this case, the removal of plants aberrant for one or several characteristics must be done before any pollen is shed.

114. In the case of using the male sterility, the inspector must be assured of the absence of male fertile or partially male fertile plants in the female parent rows.

115. In the case of mechanical emasculation for the production of maize hybrid seeds, the inspector must be assured that it is applied before the female plants have shed any pollen, and above all before the stigmas of the female plants are receptive.

116. During the field visits, the inspector must be informed by the seed grower of the harvesting conditions to ensure there will be no risk of mixing between the male and female parent. The rows of the male parent will be destroyed or harvested separately before those of the female parent. This does not apply to the production of hybrid rye (*Secale cereale*) where the male and female lines are cultivated as mixed.

117. The varietal purity of the hybrid obtained in the production crop can only be checked in a post-control plot sown with a sample of the hybrid seed produced. However, the varietal purity can be secured by ensuring that the following requirements are met:

- Adequate isolation distances from sources of contaminating pollen;
- Good conditions for pollen dissemination;
- High levels of male sterility of the female parent;
- Low levels of sibling;
- High levels of varietal purity of both parents;
- Separate harvesting or destruction of the male component ahead of the seed-bearing (female) parent.

Inspection of conformity to percentage standards

Impurities counting

118. For standards expressed as a percentage, the number of impurities observed in the sample areas has to be related to the plant population.

119. An estimate of the plant population can be obtained by counting the number of plants or ear-bearing tillers in a row of one metre length, but in the case of broadcast crops in $0.5m^2$ areas.

120. The population per hectare for crops in rows can be calculated using the following formula:

$$P = \frac{1\,000\,000\,M}{W},$$

where	P	=	the plant population per hectare
	M	=	the mean number of plants per metre length of row
	W	=	the width between rows (in centimetres)

121. For some crops, such as cereals, it is usually quicker to count fertile tillers in the form of ears or panicles rather than plants. An assumption then has to be made that each single plant will on average produce the same number of ears so that the counts obtained are proportional. The value of M is obtained by counting the number of plants or ear/panicles in a row of one metre length within each sample area and taking the mean.

122. If ears are counted rather than plants in a tillering crop, then allowance for induced heterogeneity needs to be made to both reject values and sample size. This will increase the number of ears that need to be assessed for the same level of confidence in the results. The extent of this increase depends on the number of ears per plant. In addition, the proportion of off-type ears may not be the same as the proportion of off-type plants if the number of ears per plant differs between the crop variety and the off-types (see OECD Papers - TAD/CA/S/RD(2009)7 "*Choosing a sampling scheme for cereal crop sampling*" and TAD/CA/S/RD(2010)2 "*Investigation into heterogeneity and its impact for field inspections of cereal crops*").

123. The population per hectare for broadcast crops can be calculated using the following formula:

$$P = 20\,000\ \times N,$$

where	P	=	the plant population per hectare
	N	=	the mean number of plants per 0.5 m^2

124. The value of N is obtained by counting the number of plants or ears/panicles in an area of 0.5m^2 within each sample area and taking the mean.

Sampling areas

125. The size and number of sampling areas will vary, depending on the species to be inspected; the size of the field; whether the crop is drilled or broadcast; whether it is self or cross-pollinating and the geographical area in which the crop is being grown. In practice, the National Designated Authority will determine the appropriate size and number of

sampling areas for each crop to guarantee that sufficient plants are examined in order to apply minimum standards for varietal purity.

126. If variety impurities are thought not to be evenly distributed over the field then it is better to take examine more areas of smaller size, whilst maintaining the total area sampled. This will help to ensure that the sample is more representative (see OECD Papers - TAD/CA/S/RD(2009)7 "*Choosing a sampling scheme for cereal crop sampling*" and TAD/CA/S/RD(2010)2 "*Investigation into heterogeneity and its impact for field inspections of cereal crops*")

127. The sample size depends on the nature of the crop, the category to be inspected and practical considerations. For cereals, ten sampling areas each of 10 m^2 and containing an average of 250 plants per m^2, would give a total sampling size of 25 000 plants. For other crop species this model should be followed wherever possible but may have to be adapted according to local circumstances.

128. In the case of crops sown in wide rows, the size of each sample could be a 20-25 m length of row including the space between the rows. Thus for maize, sorghum and sunflower some National Designated Authorities might consider a total of 1 000 plants to be a sufficiently large sample, while for soya bean the number could be 3 000 to 10 000; depending on the category to be inspected.

129. Where crops are broadcast, it may be possible to reduce the size of each sample area to ensure that the total number of plants examined is not more than is required statistically to give a good estimate of varietal purity.

130. In general, the number of sampling areas should increase in proportion to the size of the field. Owing to higher standards for Pre-Basic and Basic Seed crops, the number of plants examined in these categories should be larger than for Certified Seed.

131. As a general rule, a sample size of 4 x n can be used when the impurities threshold is 1 for n. Thus for a minimum varietal purity of 99.9% (1 in 1 000) the sample size should be 4 000.

132. In some crops grown for hybrid seed production, it is essential to examine all plants in the sample and to check not only for varietal purity but also that the standard for male sterility of the seed-bearing parent has been achieved.

133. For some crop species, there may be important distinguishing characters, which are described in the official description but which are too small to be examined under field conditions. These characters could be critical in the assessment of uniformity of a variety and could indicate out-pollination, segregation or mutation in the seed lot. In such circumstances, plants could be examined more easily under laboratory conditions.

134. The National Designated Authority can rely mainly on the data from the pre-control plot and use the field inspection results for confirmation only. Where there is an obvious discrepancy between the control plot and the field data, it may be necessary to conduct further examinations in both areas so that a positive decision can be made.

Reject numbers

135. For assessing crops against the standards, 'reject numbers' as described in the previous section relating to Control Plot Tests can be utilised. As stated earlier, if ears are counted rather than plants, larger reject values are required (see OECD Papers - TAD/CA/S/RD(2009)7 "*Choosing a sampling scheme for cereal crop sampling*" and

TAD/CA/S/RD(2010)2 "*Investigation into heterogeneity and its impact for field inspections of cereal crops*").

136. Some examples are given in Tables 10 and 11 for a range of standards, populations and corresponding reject numbers which might be encountered in a total sampling area of 100 square metres (10 quadrats x 10 m^2). These are applicable only when plants are counted. If ears are counted then larger reject values are required – see Tables 12 and 13.

Table 10. Plant reject numbers for a total sample area of 100 m^2 for various varietal purity standards *(99.5 to 99.9%)*

Estimated population (plants per ha)	Varietal purity standard		
	99.9%	99.7%	99.5%
	Reject number for a sample area of 100m^2 (*)		
600 000	11	26	40
900 000	15	37	57
1 200 000	19	47	74
1 500 000	23	57	90
1 800 000	26	67	107
2 100 000	30	77	123
2 400 000	33	87	139
2 700 000	37	97	155
3 000 000	40	107	171
3 300 000	44	117	187
3 600 000	47	126	203
3 900 000	51	136	219

Table 11. Plant reject numbers for a total sample area of 100 m2 for various varietal purity standards (97.0 to 99.0%)

Estimated population (plants per ha)	Varietal purity standard		
	99.0%	98.0%	97.0%
	Reject number for a sample area of 100m^2 (*)		
200 000	29	52	74
400 000	52	96	139
600 000	74	139	203
800 000**	96	182	266

137. Tables 12 and 13 below provide the ear reject numbers for various sample sizes

Table 12. Ear reject numbers for a total sample area of 100 m² or various varietal purity standards *(99.5 to 99.9%)*

Estimated population (ears per ha)	Varietal purity standard		
	99.9%	99.7%	99.5%
	Reject number for a sample area of 100m² (*)		
600 000	16	31	46
900 000	19	43	67
1 200 000	25	55	85
1 500 000	28	67	100
1 800 000	31	76	118
2 100 000	37	88	136
2 400 000	40	97	154
2 700 000	43	109	169
3 000 000	46	118	187
3 300 000	52	130	202
3 600 000	55	139	220
3 900 000	58	151	235

Table 13. Ear reject numbers for a total sample area of 100 m2 or various varietal purity standards (97.0 to 99.0%)

Estimated population (ears per ha)	Varietal purity standard		
	99.0%	98.0%	97.0%
	Reject number for a sample area of 100m^2 (*)		
200 000	34	61	82
400 000	61	106	151
600 000	85	154	220
800 000**	106	196	286

(*) Crops are rejected if the total number of impurities found in the total area of 100 m^2 is equal to or greater than the number given for the appropriate estimated population and varietal purity standard.

(**) With varietal purity levels of 99.0% and below and high plant populations in excess of 1,000,000 per ha, it is not necessary to use reject numbers. This is because the number of impurities which need to be counted to effect a rejection of the crop are so large that the difference between the expected number and the reject number is small enough to be ignored for practical purposes.

138. Species purity and varietal purity should be assessed separately and both must be satisfactory for the crop to be accepted.

Inspection of conformity to maximum number per unit area standards

139. For many crop species it is neither possible nor practical to accurately estimate plant populations because of the cropping system applied. In these cases varietal purity standards are expressed as a maximum number of impurities per unit area (see OECD Seed Schemes).

140. Following an inspection of the whole field – to check isolation conditions, limited presence of weeds, varietal identity and crop homogeneity – one of the following sampling methods described below should be used.

141. The sampling procedures are based on the following assumptions: off-type plants and plants of other species are randomly distributed throughout the crop, and the counts of impurities follow the Poisson distribution.

142. If there are patches of impurities in some parts of the field, the above assumptions become invalid. In such cases these patches should be excluded from the sampling areas and inspected separately.

143. In the design of sampling procedures when inspecting a maximum number of impurities per area standards, *the risk of making a wrong decision is biased in favour of the seed grower*, with a lower risk of the crop being wrongly rejected (α) and a greater risk that the crop might be wrongly accepted (β). This bias in favour of the seed grower can be compared to the case of crops inspected to percentage standards.

144. The two methods described below have been designed to check the conformity of the seed crop to a maximum threshold of 1 impurity per 10 m^2. Both methods accept a *20% β risk* of accepting fields for which the true level of off-type plants (or plants of other species) is 1.5 in 10 m^2, and a *less than 10% α risk* of rejecting fields with a maximum of 1.0 impurity in 10m^2.

145. Method A differs from Method B in that it allows for a maximum of two successive sets of counts (double plan). Method B is based on a sequential sampling technique with a maximum of nine successive sets.

Method A / Double plan

146. This method allows for a maximum of two steps. The field size is limited to 10 hectares with counts for impurities being made in 11 sample areas each of $10m^2$. Where the seed crop is larger than 10 hectares, the field should be sub-divided into two parts, each being inspected separately.

147. If the total number of impurities is equal to or less than 11, the field is considered to have met the minimum varietal purity standard of 1 impurity per $10m^2$. If the total is equal to or greater than 18, the standard is exceeded and the field should be rejected.

148. When the total number of impurities falls between 12 and 17, the method requires 17 additional counts to be made. If the new total of impurities obtained for the 28 sample areas is equal to or less than 35, the standard is met and the field can be approved. If it is equal to or exceeds 36, the field is rejected. The exact risks associated with this procedure are $\alpha = 0.086$ and $\beta = 0.198$.

Method B / Sequential sampling

149. This method is a sequential sampling scheme: The number of sample areas inspected is not predetermined but depends on the results of successive sampling.

150. Method B has been designed to save time, but this gain is only effective in practice when a majority of crops meets the varietal purity standard for certified seed of 1 impurity in $10m^2$. As in Method A, the field size is limited to 10 ha. Fields in excess of 10 ha should be sub-divided, with each part being inspected separately.

151. The minimum number of counts which should be made is determined by the size of the field (Table 14).

Table 14. Minimum number of counts for various sizes of field

Size of field (ha)	Number of counts
1 or 2	4
3 or 4	8
5 to 7	12
8 to 10	16

152. The total number of impurities in the counts is then assessed against the criteria for acceptance or rejection (see Table 15).

Table 15. Acceptance and rejection limits for stated numbers of counts

Number of counts	Total number of impurities	
	FIELD ACCEPTED if number is equal to or less than	FIELD REJECTED if number is equal to or greater than
4	1	10
8	6	15
12	12	19
16	18	24
20	22	30
24	27	35
28	31	39
32	36	44
36	43	44

153. If the number of impurities falls between the acceptance or rejection bands, further counts should be made (up to a maximum of 36), until a decision can be reached. The exact risks associated to this procedure are $\alpha = 0.096$ and $\beta = 0.202$.

Internationally recognized methods currently commonly used by the National Designated Authorities

154. The OECD Seed Schemes does not develop or carry out any independent testing to determine the effectiveness of laboratory techniques for the determination of varietal identity, and therefore cannot specifically endorse any specific laboratory techniques for determining varietal identity.

155. The traditional OECD field inspection techniques together with pre- and post-control plots as described in Parts I and II of the Guidelines for Control Plot Tests and Field Inspection of Seed Crops are to be regarded as the standard techniques for determining varietal identity and varietal purity.

156. However, the OECD Seed Schemes do recognise that there are occasions where these traditional techniques limit the certainty of the varietal determination, and in certain cases varieties of some species cannot be identified with certainty using these traditional techniques. In these specific circumstances, it might be beneficial to use non-field based tests, which must be seen as supplementing and not replacing the more traditional techniques.

157. The Annual Meeting of the Seed Schemes has agreed that any supplementary techniques that are used must be included in internationally recognised and documented methods. This is to ensure that any techniques used by an NDA have been shown to be accurate and repeatable.

158. The Annual meeting has therefore approved the methods listed in the annex below for inclusion in the Guidelines for Control Plot Tests and Field Inspection of Seed Crops and for use by Member Countries to support the traditional field-based techniques, only in those cases where these traditional techniques have been implemented fully but still leave some doubt as to the identity of a variety.

159. It should be noted that all characteristics that are included on the Official Description of a variety, whether they are field characters or laboratory characters, can be used by an OECD NDA for the purposes of determining varietal identity and the issue of an OECD Seed varietal Certificate.

160. These methods can be used at the discretion of the National Designated Authority, and may be applied to any category of seed.

161. Where an NDA, other than the certifying NDA, is planning to reject a seed lot based on results obtained through the use of these methods, this should be undertaken in agreement with the certifying National Designated Authority.

162. Where a technique is used that is not documented in the variety description, an authenticated standard sample of the variety must be available for comparison and must be treated and examined in the same way as the sample under test.

163. Annex A lists those internationally recognised methods that have been found reliable in identifying varieties in some member countries National Certification, in situations where the traditional field inspection and control plot techniques have been inconclusive.

Annex .A. INTERNATIONALLY RECOGNISED METHODS CURRENTLY COMMONLY USED BY THE NATIONAL DESIGNATED AUTHORITIES

The following methods were approved by the 2023 Annual meeting, and NDA's are able to use these methods for varietal identity evaluation in accordance with the Guidelines for Control Plot Tests and Field Inspection of Seed Crops in Part III. All of these methods have been used by at least 1 OECD NDA as part of their National certification programme and providing accurate results, and were in the Molecular and Biochemical survey and included in document [TAD/CA/S/RD(2016)6/REV4] " Final List of BMT Methods in use by Participating Countries", noted by the 2023 Annual Meeting.

SPECIES	NAME OF TEST	DOCUMENTED BY	REFERENCE
***Avena sativa* (Oats)**	A-PAGE (Acid-Polyacrylamide Gel Electrophoresis)	ISTA	ISTA Rule 8.8.6
	SSR based method	ISTA	2023 ISTA Rules 8.10.5 *Avena sativa* (oats) Microsatellite Markers Inclusion of DNA-based test for testing Avena sativa. Proposal supported by validation study. Proposal developed and approved by Variety TCOM.
Brassica juncea (Mustard)	Fat acid composition and erucic acid quantification	ISO	Standards NF EN ISO 17059: "Oilseeds - Extraction of oil and preparation of methyl esters of triglyceride fatty acids for analysis by gas chromatography" and NF EN ISO 12966-4: "Gas chromatography of fatty acid methyl esters — Part 4: Determination by capillary gas chromatography"
***Brassica napus* (Rapeseed)**	Isozymes in starch gels	CPVO	Annex II.2 " Description of the SGE Method (Starch Gel Electrophoreses) for the Analysis of Isoenzymes from Brassica napus " of CPVO-TP/036/2 Final " protocol for Distinctness, Uniformity and Stability tests"
	GMO detection and identification	ISTA	Internal methods validated with reference to EURL validation studies and to the standards ISO 21569 and ISO 21571
	Determination of glucosinolates content	ISO	Standard NF EN ISO 9167-1 Rapeseed and rapeseed meals — Determination of glucosinolates content — Method using high-performance liquid chromatography
	SSR Molecular Markers	ISO	ISO 13495:2013 Foodstuffs — Principles of selection and criteria of validation for varietal identification methods using specific nucleic acid
	Fat acid composition	ISO	Standards NF EN ISO 17059: "Oilseeds - Extraction of oil and preparation of methyl esters of triglyceride fatty acids for analysis by gas chromatography" and NF EN ISO 12966-4: "Gas chromatography of fatty acid methyl esters — Part 4: Determination by capillary gas chromatography"
Brassica nigra (Black mustard)	Fat acid composition and erucic acid quantification	ISO	Standards NF EN ISO 17059: "Oilseeds - Extraction of oil and preparation of methyl esters of triglyceride fatty acids for analysis by gas chromatography" and NF EN ISO 12966-4: "Gas chromatography of fatty acid methyl esters — Part 4: Determination by capillary gas chromatography"
Glycine max	Seed: coloration due to peroxidase activity in seed coat	UPOV	Ad. 16: "Seed: coloration due to peroxidase activity in seed coat" to the UPOV TG/80/6 guidelines for the conduct of tests for Distinctness, Uniformity and Stability

(soyabean)	GMO Detection, Identification and Quantification	ISTA/ EURL	Internal methods validated with reference to EURL validation studies and to the standards ISO 21569, ISO 21570 and ISO 21571
***Helianthus annuus* (Sunflower)**	SGE Method for the Analysis of Isoenzymes (Starch Gel Electrophoreses)	UPOV	Part III " SGE Method (Starch Gel Electrophoreses) for the Analysis of Isoenzymes " of the UPOVTG/81/6guidelines for the conduct of tests for Distinctness, Uniformity and Stability
	SSR based method	ISO	ISO/TR 17622:015(E)
***Hordeum vulgare* (Barley)**	A-PAGE (Acid-Polyacrylamide Gel Electrophoresis)	UPOV / ISTA	"Acid PAGE Method for Analysis of B- and C-Hordeins from *Hordeum vulgare*" in Part III "Description of the method to be used" of the UPOV tg/19/10 guidelines for the conduct of tests for distinctness, uniformity and stability / ISTA Rule 8.8.3
	SDS-PAGE (Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis)	UPOV	"Hordein composition: allele expression at loci Hor-3(30), Hor- 1(31) and Hor-2(32) - SDS PAGE Method for Analysis of Hordeins from *Hordeum vulgare*" in Part III "Description of the method to be used" of the UPOV tg/19/10 guidelines for the conduct of tests for distinctness, uniformity and stability

***Lolium* (Ryegrass)**	SDS-PAGE (Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis)	ISTA	ISTA Rule 8.8.4 for Principle & Apparatus, and for Specific Procedure: 8.8.4.3.2
Lolium multiflorum	SDS-PAGE (Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis)	ISTA	ISTA Rule 8.9.4
***Pisum sativum* (Pea)**	SDS-PAGE (Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis)	ISTA	ISTA Rule 8.8.4 for Principle & Apparatus, and for Specific Procedure: 8.8.4.3.1
	SSR based method	ISTA	2023 ISTA Rules 8.10.4 *Pisum* (peas) Microsatellite Markers Inclusion of DNA-based test for testing *Pisum* varieties. Proposal developed by working group within Variety TCOM and supported by validation study. Proposal approved by Variety TCOM.
***Solanum lycopersicum* (Tomato)**	Tomato Spotted Wild Virus resistance Sw-5 gene	UPOV/CPVO	Ad. 55: Resistance to Tomato spotted wilt virus (TSWV) of 2016 protocol (CPVO-TP/044/4 Rev2 Guidelines for the conduct of tests for DUS
Triticosecale	SDS-PAGE (Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis)	ISTA	ISTA Rule 8.8.9
	Method for Determination of Phenol Reaction	UPOV	Ad. 20 " Method for Determination of Phenol Reaction " UPOV (Ad. 25 "Method for Determination of Phenol Reaction" of UPOV TG/121/3 Guidelines for the conduct of tests for DUS)
	Seed: coloration with phenol " of the UPOV TG/121/4 Guidelines for the conduct of tests for Distinctness, Uniformity and Stability	UPOV	UPOV TG/121/4
***Triticum aestivum* (Wheat)**	SDS-PAGE (Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis)	UPOV	Part III - Description of the Method to be Used - Glutenin composition: allele expression at loci Glu-A1 (27), Glu-B1 (28) and Glu-D1 (29) - SDS PAGE Method for Analysis of HMW Glutenins from *T. aestivum* of the UPOV TG3/11 Guidelines for the conduct of tests for distinctness, homogeneity and stability

	A-PAGE (Acid-Polyacrylamide Gel Electrophoresis)	ISTA/UPOV	ISTA Rule 8.8.8 UPOV tg/19/10 guidelines for the conduct of tests for distinctness, uniformity and stability /

	Method for Determination of Phenol Reaction	UPOV	Ad 24 " Grain: coloration with phenol - Method for Determination of Phenol Reaction" UPOV TG : Ad.2 Seed coloration with phenol of UPOV TG/3/12 for Distinctness, Uniformity and Stability tests *Triticum aestivum* L. (wheat)
	Seed: coloration with phenol" of the UPOV TG/3/12 Guidelines for the conduct of tests for Distinctness, Uniformity and Stability	UPOV	UPOV TG/3/12
	GMO detection	ISO/EURL	Internal methods validated with reference to EURL validation studies and to the standards ISO 24276, ISO 21569, ISO 21570 and ISO 21571
	SRR based method	ISTA	ISTA Rule 8.10.2 Triticum (wheat) Microsatellite Markers
***Triticum durum* (Durum Wheat)**	A-PAGE (Polyacrylamide Gel Electrophoresis)	ISTA, UPOV	ISTA Rules, Chapter 8 UPOV Guidelines TG/19/10
	Method for Determination of Phenol Reaction	UPOV	Ad. 2: Seed: coloration with phenol of Ad27 TG120/4 Guidelines for the conduct of tests for DUS
***Zea mays* (Maize)**	IEF PAGE (Ultra Thin Layer – IsoElectroFocusing - Polyacrylamide Gel Electrophoresis)	ISTA	ISTA Rule 8.8.5
	GMO Detection, Identification, Quantification	ISO/EURL	Internal methods validated with reference to EURL validation studies and to the standards ISO 24276, ISO 21569, ISO 21570 and ISO 21571
	SSR based method	ISO	ISO/TR 17623:2015(E)
	A-PAGE (Polyacrylamide Gel Electrophoresis)	ISTA	ISTA Rules, Chapter 8; Poperelja, F.A. (1989) Elekroforez zeinov kukuruzy v PAGE
	SGE Method for the Analysis of Isozymes (Starch Gel Electrophoreses)	**UPOV**	Part III "Description of the SGE (Starch Gel Electrophoreses) Method for the Analysis of Isozymes from *Zea mays* L." of the UPOV TG/2/7 Guidelines for the conduct of tests for Distinctness, Uniformity and Stability
***Sorghum* (Sorghum)**	SSR Molecular Markers	ISO	ISO 13495:2013 Foodstuffs — Principles of selection and criteria of validation for varietal identification methods using specific nucleic acid

The OECD Seed Schemes are a set of procedures, measures and techniques that aim to ensure the varietal identity and purity of internationally traded seeds. The present guidelines for control plot tests and field inspection of seed crops are intended to advice National Designated Authorities on methods and techniques to determine varietal purity and identity at different stages of seed production.

The methods described have been found to provide accurate and reliable results. The guidelines have been approved by OECD member and non-Member countries that participate in the OECD Seed Schemes and exchange certified seed in international trade.

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